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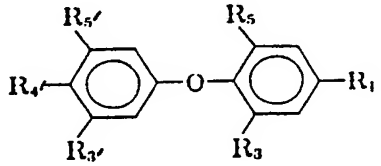

(I)

(57) Abstract

The body composition of poultry is improved by a hormonal strategy that involves either (1) increasing plasma levels of thyroid hormone by about 2- to 3-fold during the finishing phase (e.g., for chickens, 3 to 6 or 7 weeks-of-age) by providing feed containing 0.1 to 1 ppm of metabolically-active thyroid hormone of formula (I), wherein R1 is the residue of a carboxylic acid such as an alpha-amino acid or an aliphatic carboxylic acid, e.g. L-alanine, D-alanine, acetic acid, or propionic acid, R3 is iodine (I), R5 is iodine (I) or hydrogen (H), R3′ is iodine (I) or the residue of an aliphatic carboxylic acid such as butyric acid, or propionic acid, R5′ is iodine (I) or hydrogen (H), R4′ is hydroxy (OH), (2) increasing plasma levels of metabolically-active thyroid hormone by 2- to 3-fold and increasing plasma levels of growth hormone or glucagon by 2- to 10-fold for 15 to 30% of each day with any suitable method during the finishing phase of poultry growth. Marked depletion of body fat and increased body protein content are obtained with minimal loss of growth rate or efficiency of feed conversion.

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Endocrine manipulation to improve body composition of poultry

Background of the Invention

This invention relates to a method for improving the carcass quality of poultry. An aspect of this invention relates to a manipulation of the hormone system of the poultry. Still another aspect of this invention relates to means and methods for altering blood levels of hormones in the bodies of poultry, which means and methods can be employed on a commercial scale.

Description of the Prior Art

A predominant cost in intensive production of poultry is the feed energy required for metabolism and growth. The metabolizable energy derived from feedstuffs is partitioned into energy for maintenance (i.e., thermoregulation and nutrient utilization) and the energy assimilated into animal product (meat or eggs). Advances in genetics, nutrition and management have provided producers with rapidly growing poultry produced for meat (broiler chickens, turkeys, and the like) that efficiently convert feed energy and nutrients into animal product. Unfortunately, excessive fat deposition is an undesirable consequence of the accelerated growth of poultry and high nutrient density of poultry rations. As the poultry reach market age, fat deposition--rather than protein accretion--becomes the principal component of weight gain. For example in broiler chickens, body fat represents from 7 to 20% of live market weight, with abdominal fat making up about 4% of total body weight. Since accumulation of excessive body fat is considered an economic loss to both the producer and consumer of poultry meat, recent research efforts have attempted to solve the problem of excessive fat deposition in the chicken's body. The inter-dependence of nutritional and genetic factors that determine accumulation of body fat precludes a uniform strategy for nutritional restriction of fat deposition. Futhermore, genetic selection against body fat would probably reduce live market weight as well as carcass quality.

Metabolically-active agents, such as hormones, appear to have the greatest potential for manipulating fat deposition and/or

muscle development in animals raised for meat (see Kiernan et al., U.S. Patent 4,407,819 issued Oct.4, 1983). For example, injection of finishing pigs with purified porcine growth hormone (pGH) was found to increase growth rate by 10-14%, improve feed conversion by 7-19%, reduce carcass fat content by 18-25% and increase muscle mass by 24-36% (T. D. Etherton et al., J. Anim. Sci. 64:433-443, 1987). Similarly, daily administration of natural or recombinant-derived bovine GH (bGH) to dairy cows can increase milk yield by 23 to 41% (D. E. Bauman et al., J. Dairy Sci. 68:1352-1362, 1985).

In contrast, however, these discoveries are not easily applied to poultry. Daily injection of broiler chickens with natural or recombinant-derived chicken GH (cGH) does not stimulate growth; in fact, cGH treatment usually results in increased accumulation of body fat (F.C. Leung et al., Endocrinology 118:1961-1965, 1985; S.S. Liou et al., Poultry Sci. 64(Suppl. 1):136, 1985; W.H. Burke et al., Endocrinology, 1987). Apparently, endocrine regulation of growth and metabolism in domestic fowl is distinctly different from that described for food mammals since exogenous cGH treatment alone does not promote growth or improve productive efficiency. The following summary of the relevant poultry science literature provides some insight into the comlexity of the research findings in this field.

Earlier work suggested that a synthetic iodinated protein, possessing thyroxine (T_4) activity, could be used as a feed additive to increase egg production or growth rate of domestic fowl (H.W.K. Jennings, British Patent 601,469, published in May of 1948). Iodinated casein (i.e., protomone) with 1% T_4 activity was originally developed as a possible growth promoter for poultry and livestock. However, the incorporation of protomone into the feed of meat-type chickens depressed growth rate, reduced feed efficiency, lowered carcass quality, and increased mortality rate when fed throughout the growth cycle (H.R. Wilson et al., Poultry Sci. 62:811-818, 1983).

Triiodothyronine (T_3) and T_4 can be directly incorporated

into the feed of broiler chickens for the purpose of elevating serum or plasma levels of thyroid hormones (J.D. May, Poultry Sci. 59:888-892, 1980; J.D. May, in Aspects of Avian ... Implications (C.G. Scanes et al., eds.) Texas Tech Univ. Press 26:185-189, 1982). This work has shown that treatment of normal broiler chickens with 0.25 to 1 parts per million (ppm) of dietary T_3 throughout the entire growth cycle reduced body weight gain and feed efficiency. In contrast, the same doses of dietary T_4 did not impair growth performance. The depressed growth rate and reduced feed efficiency of normal (euthyroid) broiler chickens fed 1 ppm T_3 throughout the growth cycle has led to the notion that dietary T_3 is detrimental to the growth and productive efficiency of poultry.

Attempts at using administration of exogenous GH to stimulate the growth of normal chickens have generally been unsuccessful. Daily intravenous injection of thyrotropin-releasing hormone (TRH) (1 or 10 µg/kg of body weight/day) or GH-releasing factor (GRF, 10 µg/kg of body weight/day) alone or in combination for 21 days failed to stimulate growth rate or improve feed efficiency of broiler chickens despite elevated plasma GH levels (F.C. Buonomo and C.A. Baile, Dom. Anim. Endocrinol. 4:269-276, 1986). Most of the evidence for supporting the idea that exogenous GH is capable of promoting growth of broiler chickens is derived from studies on growth-compromised Leghorn (egg-type) chickens. In these studies, dwarf strains or hypophysectomized (i.e., pituitary gland surgically removed) Leghorns were given replacement doses of T_3, T_4 or GH (usually mammalian GH) alone or GH in combination with either T_3 or T_4 to determine the importance of these hormones in the normal growth process. The sex-linked dwarf Leghorn chicken has elevated plasma levels of both GH and T_4, whereas T_3 concentrations are greatly reduced. The depressed growth rate in dwarf strains of Leghorn chickens was restored to normal by supplementing their diets with either T_3 or T_4, or by the combination of T_4 with a daily injection of mammalian GH (J.A. Marsh et al., Proc. Soc. Exp. Biol. Med. 177:82-91, 1984;

and J.A. Marsh et al., Proc. Soc. Exp. Biol. Med. 175:351-360, 1984). The importance of T_3 to the normal growth process was further demonstrated by the ability of exogenous T_3, rather than GH therapy, to correct the growth deficit of hypophysectomized Leghorn chickens (C.G. Scanes et al., Growth 50:12-31, 1986). Although several studies have revealed distinct interactions between GH and the thyroid hormones in regulation of growth in chickens, this area clearly needs further research to develop a truely practical program of hormone manipulation which is useful on a commercial scale for normal, meat-type poultry.

Any hormonal treatment that restricts fat deposition while increasing carcass protein content could theoretically have a major impact on the cost and quality of poultry meat, and the formulation of poultry rations, but because poultry (particularly broiler chickens) are produced on such an enormous commercial scale, the treatment must satisfy a variety of practical criteria.

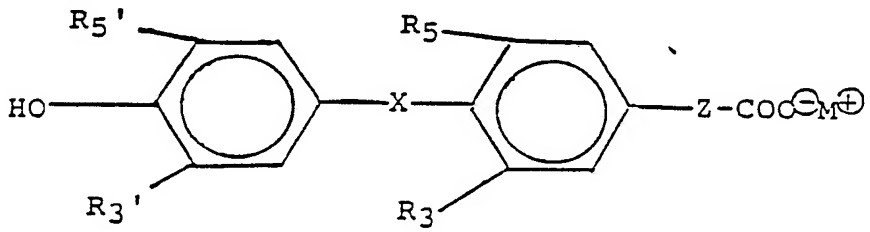
Definitions

Throughout this application, the following terms are used with the meanings indicated below.

"Finishing phase" or "finishing phase of the growth cycle" means the time period in the production of poultry after the major portion of the rapid growth of the avian species (e.g. broiler chickens and turkeys) has been completed. With modern broiler chicken production techniques, chickens grow to a high percentage of their live market weight in the first three to four weeks of life. Six or seven weeks of age is usually considered a market age for broiler chickens. Thus, the "finishing phase" for broiler chickens typically begins at about 3, 4 or (rarely) 5 weeks of age and lasts until slaughter, or a least until market age. In some embodiments of this invention, it may be desirable to permit the poultry to clear their bodies of any treatment for up to a week or so prior to slaughter. Thus, the "finishing phase" for broiler chickens can last as little as two weeks or as long as about five weeks, but in any case the rapid growth phase

has been substantially completed before the "finishing phase" is underway. For turkeys, the growth cycle lasts longer (e.g. 15 to 25 weeks), hence the "finishing phase" begins after 6 weeks of age and may last longer than four or five weeks.

"Metabolically-active thyroid hormone" refers to the natural or synthetic iodinated D- or L- or DL-thyronine compounds or iodinated phenoxyphenol-substituted aliphatic carboxylic acids having more than 15%, preferably more than 50%, of the receptor binding capability of T_3 (3,3',5-triiodo-L-thyronine, alternatively O-[4-hydroxy-3-iodophenyl]-3,5-diiodo-L-tyrosine) and preferably at least 30% of the in vivo activity of T_3. "Receptor binding" is defined herein in accordance with M.B. Bolger et al., J. Biol. Chem. 255:10271-10278 (1980). Preferred metabolically-active thyroid hormones are compounds of the formula I:



(I)

where Z is C_2-C_4 alkylene or amino-substituted C_2-C_4 alkylene;

M^+ is a physiologically acceptable cation such as H^+;

R_3 and R_5 are hydrogen or iodine, at least one of them being iodine;

R_3' and R_5' are hydrogen or iodine or $-A-COO^-M^+$, where A is C_2-C_4 alkylene and M^+ is a physiologically acceptable cation; and X is a bridging radical such as $-CH_2-$, -S- or -O- (preferably -O-); provided, that if R_3', R_5', R_3 and R_5 are all iodine (I), then $Z-COO^-$ is a residue of the anion of acetic or propionic acid. The most active compounds of formula I are T_3 itself, "Triac" ($Z=CH_2$, M=H, R_3, R_5 and $R_3'=I$, $R_5'=H$) and "Tetrac" (similar to "Triac", except that $R_5'=I$). When Z is amino-substituted, the radical $-Z-COO^-$ can be the residue of D- or L- or DL-alanine.

Brief Description of the Invention

This invention is concerned with a novel, practical hormonal treatment for poultry grown for meat production, particularly broiler chickens, that dramatically reduces carcass fat and increases carcass protein content. The treatment comprises adding an effective amount, e.g. 0.01-5 parts per million, preferably 0.1 to 1 ppm of metabolically-active thyroid hormone, preferably triiodothyronine (T_3, 3,3',5-triiodo-L-thyronine or O-[4-hydroxy-3-iodophenyl]-3,5-diiodo-L-tyrosine), to feed of broiler chickens during the finishing phase (usually 3 to 6 weeks-of-age). The consumption of feed containing 1 ppm T_3 provides broiler chickens with a 2- to 3-fold elevation of plasma T_3 levels when compared to controls. The efficacy of this invention is enhanced when, in addition to providing poultry with dietary T_3 during the finishing phase, GH (somatotropin) or glucagon levels in the bloodstream are also increased, also during the finishing phase and preferably by 2- to 10-fold. In one embodiment of this invention, poultry are provided with dietary T_3 during the finishing phase and circulating levels of glucagon relative to insulin are increased (i.e., the insulin-to-glucagon molar ratio is decreased). The timing of applying the dietary T_3 treatment alone or dietary T_3 in combination with other metabolically-active hormones (e.g. cGH or glucagon) to broiler chickens is of very great significance and should begin at the conclusion of the rapid growth phase (i.e., the start of the finishing phase) and should continue for a period of two to five weeks (i.e., until time of slaughter). There appears to be a greater advantage of applying the combination of dietary T_3 and cGH treatment. This treatment combination exerts a synergistic action, as indicated by more than 50% reduction in body fat content compared to only 17-25% reduction of body fat with dietary T_3 alone, and compared to essentially no fat reduction with cGH treatment alone. This novel manipulation of the bird's endocrine system has the advantage of dramatically reducing accumulation of excess body fat and increasing body protein content without impairing growth

rate or feed efficiency.

This invention is not bound by any theory. Available data suggest that any hormone manipulation within the scope of this invention can have an impact upon the insulin-to-glucagon molar ratio of the avian pancreatic hormonal system (the I/G molar ratio). It is presently theorized that a decrease in the I/G molar ratio of poultry during the finishing phase mobilizes body fat stores, and, if sufficient metabolically-active thyroid hormone is present, body fat content is reduced.

Detailed Description of the Invention

The above mentioned metabolically-active hormones are naturally synthesized within the body of domestic fowl and are known to be important regulators of various metabolic activities (i.e., energy, carbohydrate, lipid and protein metabolism) that contribute to normal growth and development. Within the scope of this invention, there are three major groups of metabolically-active hormones: (1) pituitary hormones [GH, prolactin and thyroid-stimulating hormone (TSH)] and their hypothalamic releasing factors [GH-releasing factor (GRF), GH-release inhibiting factor or somatostatin (SRIF), and thyrotropin-releasing hormone (TRH)], (2) the thyroid hormones (T_3 and T_4), and (3) the pancreatic hormones (insulin, glucagon and somatostatin). The natural metabolically-active hormones, synthetic analogues and their pharmacologically-acceptable salts are to be considered within the scope of this invention. Amino acid components or residues and carbohydrate components of synthetic metabolically-active substances are generally provided in the most active isomeric forms (e.g. L-amino acids, D-carbohydrates, etc.), except that racemates (DL-compounds), diastereomers, etc. can be used when at least 25% of the normal physiological activity is still obtained. Analogs of T_3 containing a D-amino acid residue can be active, apparently because of the importance of the location of other substituents on the molecules. This invention is concerned with means of enhancing circulating blood levels of

certain metabolically-active hormones such as the thyroid hormones (particulary T_3), pituitary growth hormone and/or the pancreatic hormones.

The secretion of trophic hormones from the pituitary gland is regulated by releasing or inhibiting factors secreted by the hypothalamus. Within the scope of this invention, the releasing factors that regulate secretion of TSH and pituitary GH (somatotropin) are of particular interest. Thyrotropin-releasing hormone (TRH) stimulates the release of both TSH and GH from the avian pituitary gland into the bloodstream. Under the stimulating effect of TSH, the thyroid gland predominately synthesizes and secretes T_4 (3,5,3',5'-tetra-iodothyronine) into blood. The enzymatic activity of thyroxine-5'-monodeiodinase in peripheral tissue (particularly the liver and kidney) is responsible for converting T_4 into metabolically-active T_3. The positive or stimulative pathway is represented by: TRH ---> pituitary ---> TSH ---> thyroid ---> T_4 ---> 5'-monodeiodinase activity ---> T_3. It is generally accepted that T_4 is a prohormone without significant metabolic activity and that any benefit derived from treatment of animals with exogenous T_4 is derived from its conversion, via 5'-monodeiodinase activity, into metabolically-active T_3. Thus, the attempts at stimulating the growth or productive efficiency of domestic animals (poultry and livestock) with iodinated protein (i.e., protomone) that is based on thyroxine activity [see Jennings, British Patent 601,469 dated May 6, 1948] are of questionable efficacy since thyroxine (T_4) is essentially inactive in provoking metabolic and hormonal responses. In birds, circulating T_3 levels play an important role in regulating metabolic heat production and secretion of pituitary and pancreatic hormones. It is apparent from the working Examples which follow that T_3 also regulates the secretion of insulin and glucagon from the avian pancreas. All embodiments of this invention have in common the oral (preferably dietary) administration of metabolically-active thyroid hormone (preferably T_3 or a compound of Formula I, above, which has biological

activity comparable to T_3) to poultry during the finishing phase, but not significantly prior to the finishing phase. During the finishing phase, the GH (somatotropin) naturally secreted by the poultry has already done much of its work, and there is no significant losses in body weight or protein content during this phase. There is, on the other hand, a more rapid utlization of body fat as a result of the orally-administered Formula I compound.

The other embodiments of this invention enhance or even synergize effectiveness of the metabolically-active thyroid hormone treatment by increasing blood levels of GH or by decreasing the insulin/glucagon (I/G) molar ratio. The timing of this enhancement effect need not be exactly coextensive with the metabolically-active thyroid hormone treatment, but it is believed to be useful to decrease the I/G molar ratio during the finishing phase, and insofar as GH also may depress the I/G molar ratio, GH treatment is also most useful during the finishing phase. In administring GH, it is preferred to match as closely as possible the natural timing of the pituitary release of this hormone, which follows a pattern characterized by a series of prominent peaks spaced an hour or two apart from one another. As a result, the plasma level of GH is preferably increased by 2- to 10-fold for only 15 to 30% of each day. This invention contemplates a variety of methods for elevating circulating levels of GH or enhancing secretion of GH from the avian pituitary gland. Chicken GH (cGH) can be purified from the pituitary of slaughtered birds (S. Harvey and C. G. Scanes, J. Endocrinol. 73:321-329, 1977) or produced on the commercial scale by recombinant-DNA techniques (L. M. Souza et al., J. Exp. Zool. 232:465-473, 1984: and W. H. Burke et al., Endocrinology 120:651-658, 1987). The recombinant-derived cGH differs from the naturally-occurring cGH mainly in the substitution of methionine as the N-terminal amino acid. The amino acid sequence and composition of cGH indicate that cGH shares about 77% homology with bovine GH (L.M. Souza et al., J. Exp. Zool. 232:465-473, 1984).

Chicken Growth Hormone

	10									20										30
1	T	F	P	A	M	P	L	S	N	L	F	A	N	A	V	L	R	A	Q	H
21	L	H	L	L	A	A	E	T	Y	K	E	F	E	R	T	Y	I	P	E	D
41	Q	R	Y	T	N	K	N	S	Q	A	A	F	C	Y	S	E	T	I	P	A
61	P	T	G	K	D	D	A	Q	Q	K	S	D	M	E	L	L	A	F	S	L
81	V	L	I	Q	S	W	L	T	P	V	Q	Y	L	S	K	V	F	T	N	N
101	L	V	F	G	T	S	D	R	V	F	E	K	L	K	D	L	E	E	G	I
121	Q	A	L	M	R	E	L	E	D	R	S	P	R	G	P	Q	L	L	R	P
141	T	Y	D	K	F	D	I	H	L	R	N	E	D	A	L	L	K	N	Y	G
161	L	L	S	C	F	K	K	D	L	H	K	V	E	T	Y	L	K	V	M	K
181	C	R	R	F	G	E	S	N	C	T	I									

Composition

13	ALA	A	9	GLN	Q	26	LEU	L	11	SER	S
11	ARG	R	14	GLU	E	14	LYS	K	12	THR	T
9	ASN	N	6	GLY	G	4	MET	M	1	TRP	W
11	ASP	D	4	HIS	H	11	PHE	F	8	TYR	Y
4	CYS	C	6	ILE	I	9	PRO	P	8	VAL	V

Mol. wt. = 22,225

Number of residues = 191

(L.M. Souza et al., J. Exp. Zool. 232:465-473, 1984; and W. H. Burke et al., Endocrinology 120:651-658, 1987)

Purified and recombinant-derived cGH represent exogenous (i.e., produced outside the body) forms of cGH which are subject to parenteral administration. These exogenous preparations can be given by injection or implants that provide prolonged release of cGH (see Kent et al., U.S. Patent 4,675,189 issued Jun. 23, 1987) especially during the finishing phase of the growth cycle in poultry.

Another method of enhancing blood levels of GH is by administration of the hypothalamic releasing factors that provoke endogenous GH secretion from the pituitary gland. Human GRF is a 44 amino acid polypeptide hormone with a molecular weight of 5040 (R. Guillemin et al., Science 218:585-587, 1982) that stimulates endogenous GH secretion from the pituitary gland (see Chang et al. U. S. Patent 4,562,175 issued Dec. 31, 1985). Subsequent to the isolation and characterization of human GRF, the amino

sequence has been determined for rat (J. Spiess et al., Nature 303:532-535, 1983), porcine (P. Bohlen et al., Biochem. Biophys. Res. Comm. 116:726-734, 1983), bovine (F. Esch et al., Biochem. Biophys. Res. Comm. 117:772-779, 1983), caprine, and ovine (P. Brazeau et al., Biochem. Biophys. Res. Comm. 125:606-614, 1984) forms of GRF. Apparently only the first 29 amino acids (i.e., GRF 1-29) are required for GH-releasing activity; therefore, numerous synthetic analogues have been developed that range from GRF 1-29 to GRF 1-44. Although an avian GRF has not yet been isolated and characterized, a number of these analogues possess the ability to provoke endogenous GH secretion from the chicken's pituitary (C.G. Scanes et al., Life Sci. 34:1127-1134,1984; and C.G. Scanes et al., J. Endocrinol. 108:413-416, 1986). Within the context of this invention are all the pharmaceutical acceptable salts of the natural, recombinant-derived and synthetic analogues of GRF which stimulate GH secretion from the avian pituitary gland. The route of administration of GH or GRF can be oral, parenteral or by prolonged-release implant. Instead of administering cGH, a 191 amino acid protein hormone, exogenous GRF (a polypeptide hormone ranging from 1-29 to 1-44 amino acids) can be used to increase endogenous cGH secretion.

Human Growth Hormone-Releasing Factor

	10									20										30
1	Y	A	D	A	I	F	T	N	S	Y	R	K	V	L	G	Q	L	S	A	R
21	K	L	L	Q	D	I	M	S	R	Q	Q	G	E	S	N	Q	E	R	G	A
41	R	A	R	L-NH_2																

Composition

5	ALA	A	5	GLN	Q	5	LEU	L	4	SER	S
6	ARG	R	2	GLU	E	2	LYS	K	1	THR	T
2	ASN	N	3	GLY	G	1	MET	M	2	TYR	Y
2	ASP	D	2	ILE	I	1	PHE	F	1	VAL	V

Mol. wt. = 5040

Number of residues = 44

R. Guillemin et al., Science 218:585-587, 1982

Another method of enhancing endogenous GH secretion in domestic fowl is the use of TRH--a tripeptide releasing factor (pyro-L-Glu-L-His-L-Pro-NH_2) secreted by the hypothalamus that provokes the secretion of GH, TSH and prolactin from the avian pituitary. Daily intravenous injection of 1 to 10 μg TRH/day from 4 to 6 or 8 weeks-of-age is capable of increasing the growth rate of broiler chickens (Leung et al., U.S. Patent 4,493,828 issued Jan 15, 1985). An obvious advantage of using TRH treatment as a means of enhancing GH secretion in broiler chickens is that this hypothalamic releasing factor is orally-active and can be incorporated into the feed or drinking water of poultry (Snarey et al., U.S. Patent 4,562,197, issued Dec. 31, 1985). The disadvantage of this approach of stimulating GH secretion is that TRH is a non-selective releasing factor which provokes the release of at least three pituitary hormones (i.e., TSH, GH and prolactin). Within the scope of this invention is the use of orally-active TRH applied in either the feed or drinking water of poultry to increase GH secretion during the finishing phase of the growth cycle.

Still another method of enhancing circulating blood levels of GH is the introduction of a fusion gene into somatic tissue or the germ line of poultry which leads to expression of copious amounts of GH in circulation (i.e., production of "transgenic chickens"). The microinjection of fertilized mouse ova with a hybrid fusion gene carrying the metallothionein (MT) promoter region and the structural gene which codes for either rat or human GH (i.e., a MT-GH fusion gene) results in a dramatic increase in body growth due to hypersecretion of GH (R.D. Palmiter et al., Nature 300:611-615, 1982; and R.D. Palmiter et al., Science 222:809-814, 1983). These transgenic mice typically show increases of 100- to 800-fold in serum GH levels and grow to twice the normal body size. Thus, gene insertion technology has tremendous potential for selective growth stimulation and/or improvements in productive efficiency of domestic animals. In fact, transgenic rabbits, pigs and sheep have been produced by

microinjection of the MT-GH fusion gene (R.E. Hammer et al., Nature 315:680-683, 1985). Furthermore, the introduction of a MT-GRF fusion gene into mice also results in increased body growth in the MT-GRF transgenic mice due to hypersecretion of GRF and, consequently, increased secretion of pituitary GH (R. E. Hammer et al., Nature 315:413-416, 1985). However, the nature of ovulation and fertilization of the ovum in birds does not allow microinjection of hybrid fusion genes into the fertilized ovum. Souza et al. (J. Exp. Zool. 232:465-473, 1984) have developed a recombinant retrovirus (i.e., a Rous sarcoma virus) vector that contains the entire coding region for cGH (designated SRA-cGH9). Infection of 9-day-old chicken embryos with the SRA-cGH9 retrovirus vector resulted in 3- to 10-fold increases in serum GH levels in the hatched chickens. Also within the scope of this invention is the development of transgenic chickens that carry a hybrid fusion gene for enhancing blood levels of metabolically-active hormones to be used in conjunction with dietary T_3 during the finishing phase.

Yet another method of increasing plasma GH levels is the use of neutralizing antibodies against somatostatin (SRIF) (see Maccecchini, U.S. Patent 4,599,229, issued Jul. 8, 1986). In normal pituitary function, SRIF inhibits secretion of GH and TSH from the pituitary gland; therefore, removal of the inhibitory effects of SRIF with neutralizing antibodies results in increased secretion of endogenous GH and, perhaps, TSH. Passive immunoneutralization of SRIF, achieved by injection of chickens with anti-SRIF antibodies raised in goats, sheep or rabbits, is capable of increasing plasma GH levels although body growth is not affected (G.S.G. Spencer et al., Comp. Biochem. Physiol. 85A:553-556, 1986; and F.C. Buonomo et al., Dom. Anim. Endocrinol. 4:191-200, 1987). In contrast, active immunoneutralization achieved by repeated injection (usually 3 injections made 2 to 3 weeks apart) of chickens with SRIF conjugated to a large immunogenic carrier protein (e.g., bovine serum albumin or human alpha-globulin), with the coupling agent glutaraldehyde, results

in increased growth rate. With hybridoma technology, it is now feasible to produce sufficient quantities of mouse monoclonal antibody against SRIF for commercial use. Passive immunoneutralization of SRIF in chickens could be achieved indirectly by injecting the anti-SRIF monoclonal antibody into the broiler-breeder hens which would then deposit the monoclonal antibody into the fertilized egg before oviposition occurs, or directly by injecting the monoclonal anti-SRIF antibody either into the fertilized egg before (or during) incubation or into the bird after hatching. Within the definition of the present invention is the use of active or passive immunoneutralization of SRIF to enhance endogenous GH secretion during the finishing phase of the growth cycle of poultry in combination with providing dietary T_3.

Somatostatin-14

ALA-GLY-CYS-LYS-ASN-PHE-PHE-TRP-LYS-THR-PHE-THR-SER-CYS

Mol. wt. = 1638

The final embodiment of this invention is the manipulation of the molar ratio of insulin-to-glucagon (I/G) secreted into blood by the endocrine pancreas. Endocrine regulation of metabolism in birds is distinctly different from that of mammals because glucagon is the pancreatic hormone that regulates blood glucose levels in birds, and because fat synthesis (i.e., lipogenesis) takes place in the liver of birds (R.L. Hazelwood, in Avian Physiology, P.D. Sturkie, ed., Springer-Verlag, pp. 303-325, 1986). In birds, glucagon exerts a strong catabolic action by mobilizing free fatty acids from adipose tissue (i.e., a lipolytic action) whereas insulin promotes anabolic activities (i.e., glucose uptake, the formation and storage of glycogen, etc.). Thus, the I/G molar ratio serves as the prime determinant of metabolic homeostasis in birds (R.L. Hazelwood, J. Exp. Zool. 232:647-652, 1984). A high I/G molar ratio indicates that the bird is in an anabolic mode (i.e., nutrient storage) while a low I/G molar ratio reflects the catabolic state (i.e., nutrient

utilization). The avian pancreas also produces an exceptionally large quantity of SRIF which is thought to be an important regulator of the I/G molar ratio. Of particular interest is the fact that pancreatic SRIF is a potent inhibitor of glucagon secretion in chickens; therefore, it appears that immunoneutralization of SRIF, designed to promote pituitary GH secretion, can also enhance glucagon secretion from the pancreas.

Experimentation carried out in support of this invention indicates that administration of exogenous ncGH by injection and T_3 by dietary treatment during the finishing phase of the chicken's growth cycle ultimately alters the I/G molar ratio. The metabolic events that lead to the dramatic depletion of body fat content are brought about by a reduction in the I/G molar ratio (i.e., reduced insulin and elevated glucagon levels in blood) and an increase in circulating T_3 levels. This concept is supported by the observation that dietary T_3 treatment alone depresses insulin secretion while glucagon secretion is increased (i.e., a reduced I/G molar ratio) and consequently decreases fat deposition in chickens. Treatment of chickens with propylthiouracil, a goitrogen that inhibits 5'-monodeiodinase activity and therefore the conversion of T_4 into T_3, induces a hypothyroid state that results in elevated plasma insulin levels and increased accumulation of body fat (K.L. Raheja et al., Horm. Metab. Res. 12:51-55, 1980; and Example 1 below). Furthermore, there is sufficient experimental evidence to support the idea that providing poultry with dietary T_3 and exogenous glucagon (by injection, implant or orally-active analogues of glucagon) would achieve the same benefits and improvements in body composition as the combination of dietary T_3 with any other treatment that simultaneously enhances circulating GH concentrations. This invention contemplates the use of exogenous glucagon treatment in combination with dietary T_3 as the most simple version of an endocrine manipulation designed to reduce body fat content of poultry.

Glucagon is a highly conserved polypeptide hormone which

has an identical amino acid sequence among mammals. Chicken and turkey glucagon differ from mammalian glucagon by the single substitution of serine (SER) for asparagine (ASN) at position 28 (R.L. Hazelwood, J. Exp. Zool. 232:647-652, 1986). The amino acid sequence of duck glucagon differs from other birds (chicken and turkey) due to the single substitution of threonine (THR) for serine (SER) at position 16. Because of these structural similarities, the commercial preparations of glucagon from the pancreases of slaughtered cattle and swine have the same biological and metabolic activity as endogenous glucagon when injected into chickens (see Example 3 below).

Chicken Glucagon

	10									20										30
1	H	S	Q	G	T	F	T	S	D	Y	S	K	Y	L	D	S	R	R	A	Q	D	F	V	Q	W	L	M	S	T

Composition

1	ALA	A	3	GLN	Q	2	LEU	L	2	PHE	F	1	TRP	W
2	ARG	R	1	GLY	G	1	LYS	K	5	SER	S	2	TYR	Y
3	ASP	D	1	HIS	H	1	MET	M	3	THR	T	1	VAL	V

Mol. wt. = 3,485

Number of residues = 29

R. L. Hazelwood, "Carbohydrate Metabolism", in Avian Physiology, P. D. Sturkie ed., Academic Press, pp. 303-325, 1986.

Orally-active drugs have been developed for increasing the I/G molar ratio in mammals, e.g. to combat certain mild forms of diabetes. Some of these drugs have the opposite effect in avian species; that is, they decrease the I/G molar ratio. Any agent which decreases the I/G molar ratio in birds can be substituted for exogenous glucagon treatment in this invention.

Because of the ease and convenience of administration of orally-active hormones or hormone stimulants or suppressants through poultry feed, one of the embodiments of this invention

involves a finishing feed which contains physiologically effective amounts of metabolically-active thyroid hormone (preferably T_3), alone or in combination with other orally-active compounds which stimulate or suppress hormone secretion. Finishing feeds typically contain a major amount (e.g. 60-90% by weight) of ground-up grain (corn, soybeans, etc.), a modest amount of fat (e.g. <10%), salts, vitamin and mineral premixes, amino acids, etc. The protein content is typically above 15% (e.g. 17-25% by weight), and some fiber content should be present.

Regardless of which embodiment of this invention is used, no radical changes in feed compositions or daily ration weights are necessary; indeed, conventional finishing feed formulas and amounts (except for the addition of dietary thyroid hormone and, if desired, orally active GH- or glucagon-increasing agents) are fully operative in this invention. The health of the birds does not appear to be adversely affected, and essential body functions (e.g. thermoregulation) do not appear to be adversely affected. However, economically advantageous changes in energy and/or protein content of finishing feeds are made possible by this invention.

Referring now to broiler chickens as a benchmark for the beneficial effects of this invention, it must be noted that these chickens grow from a weight of 30 to 50 grams at hatching to about 1.5-3 or even as much as 5 kilograms of body weight at market age. Of this market weight, 15-20 wt.-% is protein, 2-3 wt.-% is inorganic (showing up as ash in proximate analysis of body composition), and more than 10 wt.-% (e.g. 10-20 wt.-%) is fat, which means that the protein:fat ratio (by weight) is likely to be at or below 1.5:1 and certainly well below 2:1. In broiler chickens treated according to this invention, however, protein:-fat ratios above 1.6:1 and even above 2:1 have been obtained, due to decreases in carcass fat content exceeding 15 wt.-%. A comparable increase in the protein:fat ratio was not obtained with T_4+GH treatment (although some improvement was found); T_4 treatment alone had almost no effect upon this ratio; and various

other treatments actually seemed to decrease the protein:fat ratio at the doses tested (e.g. GH alone, TRH alone, TRH+GH, and propylthiouracil alone). In the Examples which follow, the principle and practice of this invention are illustrated. To provide maximum scientific control over the results, cGH and glucagon were administered by daily intramuscular injection even though this technique of administration would not normally be used in commercial practice. The following abbreviations are used in these Examples:

CF=control feed
T_3=3,3',5-triiodo-L-thyronine
T_4=thyroxine
PTU=propylthiouracil
TRH=thyrotropin-releasing hormone
GH=growth hormone (e.g. ncGH, natural chicken GH)
BW=body weight
ADG=average daily weight gain
ADFC=average daily feed consumption
BI=bicarbonate buffer injection
N=number of test chickens
SEM=standard error of the mean
NIC=non-injected control
BC=sodium acetate buffer-injected control
BSA=bovine serum albumin
IgG=chicken immunoglobulin G
SRIF=somatostatin or somatotropin-release inhibiting factor

Example 1

The Effect of Thyroid Manipulation and Chicken Growth Hormone Injections on Growth, Feed Efficiency and Body Composition of Broiler Cockerels

Materials and Methods

The purpose of this study was to determine the effect

manipulating blood levels of thyroid hormones and/or growth hormone on growth performance and body composition of broiler chickens. The following thyroid-active substances were purchased from Sigma Chemical Company (St. Louis, Missouri): Product T 2877 (3,3',5-triiodo-L-thyronine or T_3), Product T 2376 (3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]-L-alanine or T_4), Product P 9012 (L-pyroglutamyl-L-histidyl-L-prolinamide or TRH), and Product P 3755 (6-N-propyl-2-thiouracil or PTU). Purified natural chicken growth hormone (ncGH) was obtained from the Research and Education Center, Harbor-UCLA Medical Center, Torrance, Calif. A premix was prepared by thoroughly mixing the required quantity of thyroid-active substance (50 mg of T_3, 50 mg of T_4, 250 mg TRH) into 200 g of dextrose. The premix (200 g) was then used to prepare 50 kg batches of each experimental diet according to the formula for broiler-finisher ration described in Table 1. The diet containing 0.5% PTU was prepared by mixing 250 g of PTU into 49.75 kg of the basal ration (Table 1). The following dietary levels were thus achieved: 1 ppm T_3, 1 ppm T_4, 5 ppm TRH, 0.5% PTU, or control feed (CF).

Broiler cockerels (Ross X Arbor Acre strain) were raised to 3 weeks-of-age in a battery-brooder and then transferred to wire grow-out cages (4 birds/pen) held in two controlled-environment rooms (10 pens/room). Beginning at 3 weeks of age, eight birds (2 pens of 4 birds) were randomly assigned to each of 10 treatments. The chickens were provided the experimental diets and water ad libitum. The five dietary treatments (CF, T_3, T_4, TRH and PTU) were designated for convenience in presentation of data as Group 1. The remaining five treatments (Group 2) consisted of a dietary treatment (with the exception of PTU) plus a single daily intramuscular injection of 100 ug ncGH/kg body weight [i.e., CF + buffer injection (CF+BI), CF+GH, T_3+GH, T_4+GH, and TRH+GH]. For injection, the ncGH was reconstituted in sterile 0.025 M sodium bicarbonate buffer (pH 9.8). The 10 treatments were administered for 21 days (i.e., from 3 to 6 weeks of age).

Measurement of body weight and feed consumption at weekly intervals allowed calculation of the average daily gain (ADG,

g/bird/day), average daily feed consumption (ADFC, g/bird/day) and the feed-to-gain ratio (FTG, kg feed/kg gain) over the 21 day experimental period. Blood samples were taken each week (4, 5, and 6 weeks) just before (pre-injection) and 4 hours post-injection of ncGH. Specific radioimmunoassays were used to measure plasma levels of cGH (J.A. Proudman, Proc. Soc. Exp. Biol. Med. 175:417-419, 1984), T_3 and T_4 (L.A. Cogburn and R.M. Freeman, Gen. Comp. Endocrinol.68:113-123, 1987), insulin and glucagon (P.C. Allen and J.P. McMurtry, Poultry Sci.63:1129-1135, 1984). At the conclusion of the study, birds were killed and the carcasses frozen for proximate analysis. The frozen carcasses were ground in a meat grinder and aliquots of each ground carcass taken for determination of moisture, protein, fat and ash by established analytical procedures (Official Method of Analysis, Edition 13, W. Horwitz ed., Association of Official Analytical Chemist, Washington, D.C., 1980). Body composition data are presented as a percent of live weight at 6 weeks-of-age. Least squares regression analysis was used to test for significant differences ($P<0.05$) due treatment.

Table 1

Composition of Broiler-Finisher Ration

Ingredients	%
Corn, yellow, ground	64.88
Soybean meal, 48%	21.23
Poultry by-product meal	3.50
Corn gluten meal, 60%	4.00
Blended fat	3.31
Defluoridated phosphate	1.71
Limestone	0.47
Livestock salt (NaCl)	0.170
L-lysine	0.170
D,L-methionine	0.060
Trace mineral premix	0.050
Vitamin premix	0.050
Hormone/dextrose premix	0.400
Grand total	100%

Analysis	
Protein	20.7%
Fat	6.5%
Fiber	2.4%
Metabolizable Energy	3244 kcal/kg

Results

Growth Performance. The final body weight of hypothyroid PTU-treated chickens was 18% lower ($P<0.05$) than that of birds fed CF or thyroid hormones (Table II). Although not significantly different, TRH-fed birds had a 6% higher body weight (BW), a 9% higher ADG and a 11% higher ADFC rate than the CF group. Dietary T_3 treatment did not affect growth rate or feed efficiency of broiler chickens. In contrast, hypothyroidism induced by dietary PTU depressed growth rate and reduced feed conversion. The combination of exogenous cGH treatment with dietary T_3 or T_4 reduced the final BW, ADG and ADFC by 12 to 15% when compared to the CF+BI group (Table III). Compared to the FTG ratio of the CF+BI group, feed efficiency was improved ($P<0.05$) by 9% in the CF+GH group and by 5% in the TRH+GH treatment.

Table II

Growth and Feed Efficiency of Broiler Cockerels Fed Thyroid-active Substances (Group 1)

Treatment	BW (kg)	ADG	ADFC	FTG
CF	$1.74\pm.01^{a}$	52.0 ± 2.1^{ab}	112.9 ± 0.7^{ab}	$2.18\pm.10^{ab}$
1 ppm T_3	$1.70\pm.09^{a}$	48.8 ± 4.3^{b}	101.1 ± 5.2^{b}	$2.08\pm.08^{b}$
1 ppm T_4	$1.76\pm.04^{a}$	51.9 ± 3.3^{ab}	108.4 ± 3.6^{b}	$2.09\pm.06^{b}$
5 ppm TRH	$1.85\pm.08^{a}$	56.5 ± 0.2^{a}	125.2 ± 5.5^{a}	$2.21\pm.01^{ab}$
0.5% PTU	$1.42\pm.05^{b}$	39.2 ± 4.0^{c}	92.3 ± 7.9^{c}	$2.36\pm.04^{a}$

Means (±SEM) within a column possessing a different superscript are significantly ($P<0.05$) different.

Table III

Growth and Feed Efficiency of Broiler Cockerels Fed Thyroid Active Substances and Injected Daily with ncGH (Group 2)

Treatment	BW (kg)	ADG	ADFC	FTG
CF + BI	1.77±.04^{a}	53.3±1.3^{a}	118.4±1.2^{a}	2.22±.08^{a}
CF + GH	1.83±.04^{a}	57.0±0.4^{a}	115.8±1.8^{ab}	2.03±.02^{c}
T_3 + GH	1.58±.06^{b}	46.2±3.9^{b}	102.6±6.7^{bc}	2.22±.04^{a}
T_4 + GH	1.54±.07^{b}	44.8±0.6^{b}	97.7±1.9^{c}	2.18±.01^{ab}
TRH + GH	1.75±.07^{ab}	53.5±0.3^{a}	113.3±1.2^{ab}	2.12±.01^{b}

Means (±SEM) within a column possessing a different superscript are significantly ($P<0.05$) different.

Body Composition. The PTU treatment increased ($P<0.05$) body fat content by 50% at the expense of body ash, protein and water when compared to the CF group (Table IV). In contrast, dietary T_3 alone reduced body fat content by 17% while body protein and water were slightly increased. Dietary TRH increased body fat content by 12% although not significantly different from the CF birds. Dietary T_3 plus exogenous cGH treatment reduced ($P<0.05$) body fat content by 51% while the ash, protein and water contents were increased by 6 to 9% above that of the CF+BI group (Table V). The combination of dietary T_4 and daily cGH injection improved body composition since body fat was reduced ($P<0.05$) by 26% when compared to the CF+BI group.

Table IV

Body Composition of Broiler Chickens Fed Thyroid-active Substances (Group 1)

		% BW			
Treatment	N	Water	Protein	Fat	Ash
CF	8	66.4±.54^{b}	17.8±.46^{a}	12.1±.67^{bc}	2.32±.02^{ab}
T_3	8	68.6±1.0^{a}	18.4±.22^{a}	10.1±1.2^{c}	2.19±.05^{bc}
T_4	8	65.8±.29^{b}	18.2±.31^{a}	12.9±.41^{bc}	2.35±.03^{a}
TRH	8	65.5±.73^{b}	18.1±.21^{a}	13.6±.88^{b}	2.34±.06^{ab}
PTU	8	61.9±.73^{c}	17.2±.20^{b}	18.2±.80^{a}	2.17±.06^{c}

Means (±SEM) within a column possessing a different superscript are significantly ($P<0.05$) different.

Table V

Body Composition of Broiler Chickens Fed Thyroid-active Substances and Injected Daily with ncGH (Group 2)

		%BW			
Treatment	N	Water	Protein	Fat	Ash
CF + BI	8	64.7±.32^{c}	18.3±.18^{b}	14.6±.47^{a}	2.34±.05^{ab}
CF + GH	8	64.7±.51^{c}	18.1±.15^{b}	14.5±.52^{a}	2.20±.04^{b}
T_3 + GH	8	70.4±.46^{a}	19.3±.12^{a}	7.1±.57^{c}	2.51±.04^{a}
T_4 + GH	8	67.3±.46^{b}	18.6±.19^{b}	10.8±.57^{b}	2.47±.07^{ab}
TRH + GH	8	65.9±.62^{bc}	18.4±.11^{b}	13.0±.66^{a}	2.22±.06^{b}

Means (±SEM) within a column possessing a different superscript are significantly ($P<0.05$) different.

Plasma Hormone Concentrations. The average plasma T_3 level of T_3-fed birds was 2.6-times higher ($P<0.05$) than that of birds in the CF, TRH or T_4 treatments (3 ng/ml) (Group 1). In contrast, the average T_3 level in the PTU-treated birds (1.5 ng/ml) was 53% lower ($P<0.05$) than the CF birds. Compared to the average of CF and TRH treatments, plasma T_4 levels were 9.6-times higher ($P<0.05$) in T_4-fed birds and reduced ($P<0.05$) by 58% in T_3-fed birds and by 76% in PTU-fed birds. Plasma GH levels were 1.9-times higher in PTU-fed birds and 32% lower in T_3-fed birds compared to the CF treatment. The plasma insulin/glucagon (I/G) molar ratio (i.e., increased glucagon and reduced insulin levels) of T_3-fed birds was 4.3-times lower ($P<0.05$) than that of CF birds (2.18) (Table VI). In contrast, the I/G molar ratio of PTU-treated birds was 2.9-times greater ($P<0.05$) than that of the CF group.

In Group 2, the average plasma GH concentration at 4 hours post-injection of 100 µg ncGH/kg BW (62 ng/ml) was 3-times higher ($P<0.05$) than the pre-injection GH concentration. The combination of daily cGH injection with dietary thyroid hormone reduced ($P<0.05$) the plasma I/G molar ratio by 6.8-fold in T_3-fed birds and by 2.2-fold in T_4-fed birds (Table VII). Clearly, these data indicate that elevated plasma T_3 levels inhibit insulin secretion whereas glucagon secretion is enhanced. The simultaneous elevat-

tion of cGH and T_3 levels in plasma potentiate this effect and lead to a dramatic reduction in deposition of body fat in broiler chickens.

Table VI

Plasma Concentration of Pancreatic Hormones in Broiler Chickens Fed Thyroid-active Substances (Group 1)

		-------- pg/ml ---------		
Treatment	N	Insulin (I)	Glucagon (G)	I/G Molar Ratio
CF	24	1038^{b}	289^{ab}	2.18^{b}
T_3	24	409^{c}	491^{a}	0.51^{c}
T_4	24	776^{bc}	318^{ab}	1.49^{bc}
TRH	24	814^{bc}	295^{ab}	1.68^{bc}
PTU	24	2349^{a}	225^{b}	6.35^{a}

The I/G molar ratio was calculated from plasma insulin and glucagon levels in each plasma sample assuming molecular weights of 5734 for insulin and 3485 for glucagon.

Means (±SEM) within a column possessing a different superscript are significantly ($P<0.05$) different.

Table VII

Plasma Concentration of Pancreatic Hormones in Broiler Chickens Fed Thyroid-active Substances and Injected Daily with ncGH (Group 2)

		---------- pg/ml ----------		
Treatment	N	Insulin (I)	Glucagon (G)	I/G Molar Ratio
CF + BI	24	1323^{a}	313^{b}	2.57^{a}
CF + GH	24	1107^{b}	298^{b}	2.28^{a}
T_3 + GH	24	249^{d}	410^{a}	0.38^{c}
T_4 + GH	24	598^{c}	314^{b}	1.19^{b}
TRH + GH	24	1047^{b}	338^{ab}	1.92^{a}

Means (±SEM) within a column possessing a different superscript are significantly ($P<0.05$) different.

Example 2

The Effect of Dietary T_3 and ncGH Injection on Growth, Feed Efficiency, and Body Composition of Broiler Cockerels

Materials and Methods

The purpose of this study was to confirm the original finding of a synergism between exogenous cGH and dietary T_3 in reducing deposition of body fat in broiler chickens (Example 1). Forty-eight 3-week-old broiler cockerels (Ross X Arbor Acre) were randomly divided into four treatment groups that contained three pens of four birds. The birds were housed in a controlled-environment room under a 20 hour light/4 hour dark cycle with feed and water provided ad libitum. The four treatment groups were: control feed (CF)+buffer injection (BI), 1 ppm dietary T_3+BI, CF+GH injection (100 µg/kg BW/day), and 1 ppm dietary T_3+GH injection (100 µg/kg BW/day. The basal feed ration was formulated according to the ingredient composition in Table I.

The birds were continuously provided with CF or feed containing 1 ppm T_3 from 3 to 6 weeks-of-age. Each bird in the CF+GH and T_3+GH treatment groups received a single intramuscular injection of 100 µg ncGH/kg BW each day for 21 days. The preparation of ncGH used in this study was from the same lot used in Example 1. Birds in the CF+BI and T_3+BI treatments received a single intramuscular injection of 0.5 ml 0.025M sodium bicarbonate buffer (pH 9.8) each day for 21 days.

Body weights and feed consumption was determined at weekly intervals although the ADG, ADFC and FTG ratio was determined over the 21 day period. Blood samples were taken each week (4, 5 and 6 weeks-of-age) just before (pre-injection) and four hours post-injection of ncGH. At the conclusion of the study (6 weeks-of-age), birds were killed and the abdominal fat removed and weighed. The liver and abdominal fat were returned to the carcass which was frozen for proximate analysis as described in Example 1.

Results

The ADG, ADFC and FTG ratio was not affected by dietary T_3, daily ncGH injection or the combination of T_3+GH treatments

(Table VIII). There was no significant effect of treatment on either the final (6 week) body weight or the relative liver weight (Table IX-A). However, dietary T_3 treatment alone reduced ($P<0.05$) the abdominal fat content by 28% whereas the combination of T_3+GH treatments was twice ($P<0.05$) as effective in reducing abdominal fat content (i.e., a 55% reduction). Daily injection of ncGH alone (CF+GH) did not affect growth performance, final body weight, relative liver weight or abdominal fat content. Body fat content (%BW) was reduced by 16% in T_3-fed birds and by 30% in birds given the T_3+GH treatment combination (Table IX-B). The body water and ash contents were also increased in birds treated with T_3 alone or in combination with GH injection.

The average plasma GH concentration at 4 hours post-injection of 100 µg ncGH/kg BW at 4, 5 and 6 weeks-of-age was 4-times higher than the pre-injection plasma GH levels. The average plasma T_3 level in the T_3+BI and T_3+GH treatment groups was 2.3-times higher than that of birds given the CF+BI or CF+GH treatment.

Table VIII

Growth and Feed Efficiency of Broiler Chickens Fed Triiodothyronine (T_3) and Injected Daily with ncGH

Treatment	N	ADG	ADFC	FTG
CF + BI	3	53.5±0.5	118.5±1.8	2.21±.02
T_3 + BI	3	52.6±1.0	117.9±0.8	2.24±.03
CF + GH	3	53.0±2.8	115.4±3.5	2.18±.05
T_3 + GH	3	49.5±4.1	111.2±6.0	2.26±.07

Each value represents the mean (±SEM) of three pens (4 birds/pen) over the three week experimental period (e.g., N=3).

Table IX-A

Final Body Weight and Relative Weight (%BW) of the Liver and Abdominal Fat of Broiler Cockerels Fed Triiodothyronine (T_3) and Injected Daily with ncGH

Treatment	N	Body Weight (BW, kg)	Liver (%BW)	Abdominal Fat (%BW)
CF + BI	12	1.86±.060	2.91±.239	2.57±.129[a]
T_3 + BI	12	1.86±.048	2.54±.096	1.86±.396[b]
CF + GH	12	1.88±.051	2.74±.102	2.56±.178[a]
T_3 + GH	12	1.77±.071	2.80±.059	1.16±.143[c]

Means (±SEM) within a column possessing a different superscript are significantly ($P<0.05$) different.

Table IX-B

Body Composition of Broiler Cockerels Fed Triiodothyronine (T_3) and Injected Daily with ncGH

		%BW			
Treatment	N	Water	Protein	Fat	Ash
CF + BI	12	63.5±.34[c]	17.2±.29	15.7±.33[a]	2.31±.073[ab]
T_3 + BI	12	65.5±.60[b]	17.3±.36	13.2±.62[b]	2.40±.054[a]
CF + GH	12	63.7±.33[bc]	17.0±.17	15.9±.32[a]	2.23±.058[b]
T_3 + GH	12	67.7±.46[a]	17.2±.16	11.0±.54[c]	2.39±.028[a]

Means (±SEM) within a column possessing a different superscript are significantly ($P<0.05$) different.

Example 3

Effect of Daily Injection of Glucagon on Growth, Feed Efficiency and Body Composition Of Broiler Cockerels

Materials and Methods

The purpose of this experiment was to determine if treatment of broiler chickens with exogenous glucagon alone would affect the growth performance or body composition. Crystalline bovine/porcine glucagon was obtained from Sigma Chemical Co. (Product G 4250) and further purified by high-performance liquid chromatography (HPLC) for injection. The glucagon was dissolved in

sterile 0.02M sodium acetate buffer (pH 5.5) containing 1.6% glycerin.

Thirty-two 4-week-old broiler cockerels (Ross X Arbor Acre) were randomly assigned to eight pens containing four birds each. The four treatments were: non-injected control (NIC), sodium acetate buffer-injected control (BC), 125 μg/kg BW twice/day (or 250 μg/kg BW/day), and 250 ug/kg BW twice/day (or 500 μg/kg BW/day) for 14 days (i.e., 4 to 6 weeks-of-age). The first injection was given between 1000 to 1100 hour while the second injection of the day was given between 1400 to 1500 hour. Blood samples were taken just before the second daily injection (pre-injection) and 30 minutes post-injection at 5 and 6 weeks-of-age. The measurements of growth performance and body composition were the same as those in Example 1.

Results

Two daily injections of glucagon did not affect the final (6 weeks) body weight, ADG or ADFC of broiler chickens (Table X). The highest dose of glucagon (500 μg/kg/day) reduced ($P<0.05$) feed efficiency as indicated by a 9% increase in the FTG ratio. The relative liver weight was increased by 30% in the 250 μg/kg BW/day group and by 53% in the 500 μg/kg BW/day group when compared to that of the BC group (2.49% BW). When compared to the BC group, the highest daily dose of glucagon (500 μg/kg) increased ($P<0.05$) the body fat content of broiler chickens by 12% (Table XI). Daily injections of glucagon increased plasma levels of free fatty acids (an index of lipolysis) by 4- to 6-fold at 30 minutes post-injection. However, the net effect of glucagon treatment was increased accumulation of body fat. There was no effect of glucagon treatment on plasma levels of GH, T_3 or T_4. Therefore, these data indicate that glucagon treatment per se can not be used to reduce body fat content of chickens although increased glucagon secretion is apparent in chickens fed T_3 alone or T_3 in combination with daily injection of ncGH (Example 1). However, the combination of dietary T_3 with exogenous glucagon can also reduce the body fat content of poultry.

Table X

Growth and Feed Efficiency of Broiler Cockerels Given Two Daily Injections of Glucagon[1]

Treatment	BW (kg)	ADG	ADFC	FTG
NIC	2.27±.07	75.3±1.8	159.5±0.9	2.12±.01^{b}
BC	2.18±.06	68.0±3.8	147.3±9.5	2.16±.02^{b}
250 µg/kg/day	2.33±.06	76.6±1.0	166.9±1.4	2.18±.05^{ab}
500 µg/kg/day	2.20±.05	69.2±3.6	162.9±5.3	2.36±.05^{a}

[1]The four treatment groups were non-injected controls (NIC), buffer-injected controls (BC), 125 µg/kg twice a day (250 µg/kg/day), and 250 ug/kg twice a day (500 µg/kg/day).

Means (±SEM) within a column possessing a different superscript are significantly (P<0.05) different.

Table XI

Body Composition of Broiler Cockerels Given Two Daily Injections of Glucagon

		%BW			
Treatment	N	Water	Protein	Fat	Ash
NIC	8	61.2±.59	17.2±.17	18.2±.68^{ab}	2.29±.08
BC	8	62.3±.76	17.4±.16	17.4±.76^{b}	2.30±.06
250 µg/day	8	61.5±.30	17.1±.11	18.6±.30^{ab}	2.16±.07
500 µg/day	8	60.6±.64	16.9±.22	19.4±.44^{a}	2.17±.03

Body composition is expressed as a percent of final body weight (%BW).

Means (±SEM) within a column possessing a different superscript are significantly (P<0.05) different.

Example 4

Effect of Active Somatostatin Immunoneutralization on Growth Rate and Abdominal Fat of Broiler Cockerels

Materials and Methods

The purpose of this experiment was to determine if active

immunization of chickens against somatostatin (SRIF) conjugated to either bovine serum albumin (BSA) or chicken immunoglobulin G (IgG) would affect growth rate, abdominal fat content or plasma GH levels of broiler chickens. The coupling agent used to conjugate SRIF to either BSA or chicken IgG was 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (ECDI) (Product E 7750, Sigma).

Two grams of BSA (Product A 7888, Sigma) were dissolved in 10 ml of 0.05 M phosphate buffer pH 7.0 (200 mg/ml). Five milligrams (mg) of SRIF (Product S 9129, Sigma) were dissolved in 1 ml of the phosphate buffer (5 mg/ml). The ECDI was prepared by dissolving 2 g of ECDI in 5 ml of 0.05 M phosphate buffer. The conjugation was achieved by first adding 4 mg of SRIF (0.8 ml) and 10 mg of BSA (0.05 ml) to a 10 ml polypropylene vial and then 10 mg of ECDI (0.025 ml) was added. The mixture was stirred at room temperature for 30 minutes and an additional 0.025 ml of ECDI was added. Following an additional 30 minutes, 2.1 ml of phosphate buffer was added to the reaction vial and the contents transferred to dialysis tubing with a molecular weight cut-off of 3500. The SRIF-BSA conjugate was dialyzed against 550 ml of 0.05M phosphate buffer at 4°C for 48 hours. The optical density of the dialysate was measured at 280 nm to determine the concentration of unconjugated SRIF removed by dialysis. Approximately 1.9 mg of SRIF was conjugated to 10 mg of BSA.

Ten milligrams of chicken IgG (Cappel Product 6004-0080, Organon Teknika Corp., Malvern, Penn.) and 5 mg of SRIF (Sigma) were dissolved in 1.1 ml of 0.05M phosphate buffer in a 10 ml polypropylene vial. Then, 10 mg of ECDI (0.025 ml) were added and the solution was mixed at room temperature for 30 min; an additional 10 mg of ECDI was then added. Following the second 30 minute reaction time, 1.85 ml of phosphate buffer was added and the contents transfer to dialysis tubing (3500 molecular weight cut-off). The SRIF-IgG conjugate was dialyzed against 600 ml of 0.05M phosphate buffer for 48 hours at 4°C. Measurement of the optical density of the dialysate at 280 nm allowed calculation of unconjugated SRIF by the extinction coefficient method. Approximately 2.65 mg of SRIF was conjugated to 10 mg of chicken IgG.

The dosage of conjugate to be used for the immunization studies were based on 0.10 mg SRIF content in the conjugate. The

immunogen was prepared by diluting 600 μg of each SRIF conjugate (SRIF-BSA or SRIF-IgG) to a final volume of 3 ml which was then emulsified with 3.0 ml of Freund's complete adjuvant. The control immunogen (BSA) was prepared by emulsifying 3.2 mg of BSA (3 ml) with 3 ml of Freund's complete adjuvant. Fifteen 4-week-old broiler cockerels were randomly divided into three groups or pens (BSA-Control, SRIF-BSA and SRIF-IgG treatments). Each bird was injected with 1 ml of the emulsion, containing 0.10 mg SRIF peptide in the conjugate for the SRIF-BSA and SRIF-IgG groups and 0.53 mg BSA for the BSA-Control group, given at two intramuscular sites and one subcutaneous site. At 6 weeks-of-age, each bird was given a 1 ml booster immunization containing 0.10 mg of SRIF (SRIF-BSA or SRIF-IgG) or 0.53 mg BSA (BSA-Control group) emulsified in Freund's incomplete adjuvant. Body weights and feed consumption per pen of five birds were determined at weekly intervals for four weeks (i.e., until 8 weeks-of-age). A 5 ml blood sample was obtained from each bird at 6, 7 and 8 weeks-of-age for measurement of plasma GH levels. At 8 weeks-of-age, the birds were killed and the abdominal fat removed and weighed.

Results

Table XII

Final Body Weight and Relative Weight of Abdominal Fat in Broiler Chickens Actively Immunized Against Somatostatin

Treatment	N	Final BW (kg)	Abdominal Fat (%BW)
BSA-Control	5	3.19	2.44
SRIF-BSA	5	3.12	3.04
SRIF-IgG	5	3.34	2.81

Final body weight (BW) was determined at 8 weeks of age.

Table XIII

Plasma Growth Hormone Levels (ng/ml) in Broiler Chickens Actively Immunized Against Somatostatin

		Age (wk)		
Treatment	N	6	7	8
BSA-Control	5	15.4	9.2	6.5
SRIF-BSA	5	17.7	14.8	11.9
SRIF-IgG	5	28.2	23.9	13.4

These results are believed to demonstrate that: (1) immunoneutralization of SRIF can provide dramatic increases in endogenous GH levels; (2) increased plasma GH levels alone can increase, rather than decrease, the fat content, unless the available metabolically-active thyroid hormone is adequate to provide increased metabolism or utilization of body fat; cf. Tables V and IX above; (3) SRIF-IgG is a superior conjugate for immunoneutralization of endogenous SRIF; and (4) ECDI is a superior coupling agent.

What is claimed is:

1. A method for lowering the extent of fat deposition in living poultry having normal or enhanced pituitary function during the normal growth cycle of the poultry, without detracting from the growth rate, which comprises:

providing exogenous metabolically-active thyroid hormone to the living poultry during the finishing phase of the normal growth cycle of the poultry, said providing of the exogenous metabolically-active thyroid hormone being delayed until the poultry are at least about 3 weeks of age.

2. A method according to claim 1 wherein the metabolically-active thyroid hormone is exogenous 3,3',5-triiodo-L-thyronine, and the said hormone is orally administered to the poultry.

3. A method according to claim 2, wherein the said hormone is fed to poultry in the finishing feed formula, in the amount of about 10 parts per billion to about 5 parts per million, based on the weight of a daily ration of feed.

4. A method according to claim 3, wherein said amount is about 0.1 to 2 parts per million, on the same basis.

5. A method according to claim 1, wherein the poultry are provided with an enhanced blood level of growth hormone, also during the finishing phase of the normal growth cycle.

6. A method according to claim 5, wherein the increased blood level of endogenous growth hormone is obtained by suppressing the inhibitory effect of somatostatin upon the avian pituitary gland.

7. A method according to claim 6, wherein the endogenous somatostatin in the poultry is immunoneutralized.

8. A method according to claim 1, wherein the poultry are provided with an enhanced blood level of glucagon, also during the finishing phase of the normal growth cycle.

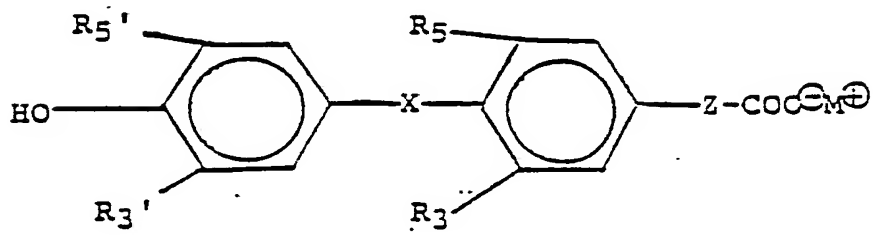
9. A method according to claim 1, wherein the poultry are treated in accordance with the said method for a least about three to five weeks.

10. A method according to claim 9 wherein the poultry are broiler chickens grown substantially for meat production, wherein the body fat content of the broiler chicken is decreased as a result of said method by at least 15% by weight, compared to untreated broiler chickens.

11. A method according to claim 1, wherein the poultry are provided with a decrease in the insulin-to-glucagon molar ratio during the finishing phase of the normal growth cycle of the poultry.

12. A method for lowering the extent of fat deposition in living poultry having normal or enhanced pituitary function, during the normal growth cycle of the poultry, without detracting from the normal growth rate, which comprises:

(a) waiting until the poultry are more than two weeks of age and then feeding to the poultry a finishing feed formula containing from about 10 parts per billion to about 5 part per million, based on the weight of the feed, of a metabolically-active thyroid hormone of the formula



(I)

wherein X is O, S, or CH_2,

Z is C_2-C_4 alkylene or amino-substituted C_2-C_4 alkylene,
M^+ is a physiologically acceptable cation,
R_3 and R_5 are H or iodo, at least one of them being iodo,
R_3' and R_5' are iodo, or hydrogen or -A-COO-M^+, where A is C_2-C_4 alkylene and M^+ is a physiologically acceptable cation,

provided, that when R_3', R_5', R_3 and R_5 are all iodo, then $Z\text{-}COO^-$ is the residue of the anion of acetic or propionic acid;

said thyroid hormone having at least about 50% of the activity of 3, 3', 5-triiodo-L-thyronine;
(b) immunoneutralizing the endogenous somatostatin of the poultry

13. A method according to claim 12, wherein the immunoneutralizing of the somatostatin is carried out by passive immunization with monoclonal or polyclonal somatostatin-inhibiting antibodies.

14. A method according to claim 12, wherein the immunoneutralizing of the somatostatin is carried out by active immunization with a somatostatin-chicken immunoglobulin G conjugate administered prior to or at the outset of the finishing phase of the normal growth cycle of the poultry.

15. A method according to claim 14, wherein the somatostatin-chicken immunoglobulin G conjugate has been conjugated with a carbodiimide conjugating agent.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 89/01018

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4): A61K 35/55 37/36

U.S.Cl. 424/111,85 514/2,21,567

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/111,85; 514/2,21,567

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

CAS, BIOSIS, APS

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Reprod. Nutr. Develop., Volume 27, No. 2B, issued 1987, E. Decuypere, J. Buyse, C.G. Scanes and E.R. Huhn, "Effects of hyper- or hypothyroid status on growth, adiposity and levels of growth hormone, somatomedin C and thyroid metabolism in broiler chickens," pages 555-565. See pages 557 and 558.	1-35
Y	Aspects of Avian Endocrinology: Practical and Theoretical Implications, Volume 26, issued 1982, J.D. May, "Effect of dietary thyroid hormones on serum hormone concentration, growth and body composition of chickens", pages 185-189. See pages 187-189.	1-35
Y	J. Endocr., Volume 96, issued 1983, S. Harvey, "Thyroid hormones inhibit growth hormone secretion in domestic fowl (Gallus domesticus)", pages 329-334. See page 332.	1-35

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24 May 1989	12 JUL 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Jean C. Witz

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	General and Comparative Endocrinology, Volume 59, issued 1985, F.C. Leung, J.E. Taylor, and A. VanIderstine, "Effects of dietary throid hormones on growth, plasma T_3 and T_4, and growth hormone in normal and hypothyroid chickens," pages 91-99. See pages 93-95.	1-35
Y	Chemical Abstracts, Volume 100, No. 21, issued 21 May 1984 (Columbus, Ohio, USA), Pamela A. Stewart and Kenneth W. Washburn, "Variation in growth hormone, triiodothyronine (T_3) and lipogenic enzyme activity in broiler strains differing in growth and fatness," see page 377, column 2, the abstract No. 171797c, Growth 1983, 47(4), 411-25 (Eng.).	1-35